eurofins | Transplant Genomics

HOW TRAC ID IS REDEFINING:

TRANSPLANT SUCCESS

SUCCESS RATES FOR ORGAN TRANSPLANTS OF ALL TYPES HAVE INCREASED SUBSTANTIALLY IN THE PAST HALF-CENTURY.

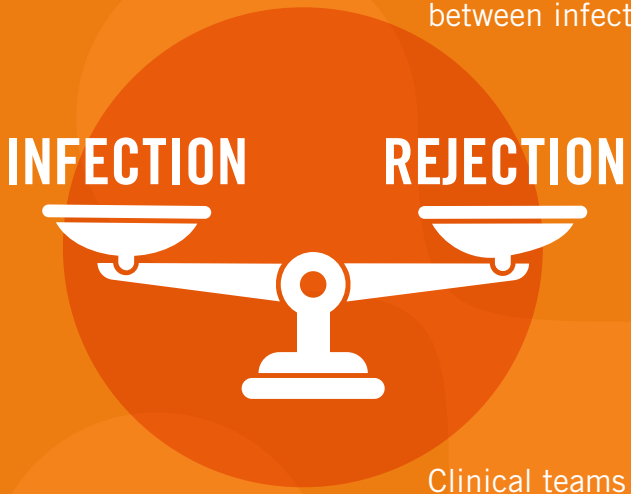
In the 1970s, renal transplant patients aged 35 and up had a **one-year survival** rate of around 60%. By the 1990s, survival rates were almost 90%. Today, **national one-year survival rates for kidney transplant** patients are above 97%.

Continuous innovations in transplant have contributed greatly to raising organ transplant success and long-term viability rates over the decades. The need to balance immunosuppression to prevent rejection while supporting the body's natural infection response has become increasingly clear. However, traditional methods of monitoring have fallen short of the precision required for tailored approaches to care.

With biopsies as the long-held gold standard for decision-making and monitoring, clinical teams and patients need less expensive, more convenient, non-invasive monitoring options to help improve transplant outcomes.

CURRENT CHALLENGES IN TRANSPLANT MONITORING

Dr. Steve Kleiboeker, the Vice President of Research and Development at Eurofins Transplant Genomics, calls the work done by clinical teams after an organ transplant a "constant balancing act between infection and rejection."



"Often, you can solve one problem, but you greatly elevate the risk of the opposite problem," says Dr. Kleiboeker. "Solving the rejection means you use a lot of immunosuppressants, and that's great until your patients have serious complications with viruses and other opportunistic infections."

Clinical teams working with kidney or liver transplants or other post-transplant situations must determine the appropriate level of immunosuppression for each case. If there is too little immunosuppression, the patient may experience rejection. The consequences can be dire: a rejection not treated quickly can cause graft failure or patient death. Excessive immunosuppression also poses serious risks, as a lack of immune response can lead to infections. The worst-case scenarios may be fatal.

POST-TRANSPLANT INFECTION RISKS:

FROM THE FIRST MONTH TO OVER A YEAR

Donor-derived viruses are typically the biggest concern in the tenuous month following a transplant. However, clinical teams must continue to closely monitor infection risks in the first 1 to 2 years. Activation of latent infections, residual or relapse viruses, and opportunistic infections pose high risks in the first 12 months. Beyond that, community-acquired infections remain a constant concern.

Dr. Kleiboeker notes that common infection risks for post-transplant patients include adenovirus, BK polyomavirus, cytomegalovirus, some forms of hepatitis, community-acquired respiratory viruses, and Epstein-Barr virus.

The rate of infections in post-transplant patients is significant. In a clinical study of almost 2,000 samples from post-transplant patients, researchers found that more than 50% tested positive for at least one virus. Among the samples that tested positive, around 25% tested positive for more than one virus.

THE HIGH OCCURENCES OF OVER-IMMUNOSUPPRESSION

Historically, post-transplant care has been a trial-and-error process with a constantly moving target, as all patients are different. In this environment, over-immunosuppression occurs regularly as clinical teams take a better-safe-than-sorry approach to reducing rejection. Subclinical rejection can occur to a degree that causes significant damage even before symptoms are reported or traditional monitoring methods detect it. This causes teams to be somewhat aggressive in their approach.

In addition to increasing infection risks, over-immunosuppression can lead to other negative outcomes, including an increased potential for malignancies. Dr. Kleiboeker points out that over-immunosuppression to protect graft health can result in patients dying with fully functional grafts due to other complications. Among cases where a patient dies with a functional graft, the causes include:

Malignancy	**20%**
Infection	**20%**
Cardiac Issues	**12%**
Other	**11%**
Not Known	**37%**

A more precise approach to individualized immunosuppression needs can help avoid some of these outcomes.

INTEGRATING MOLECULAR PANEL TECHNOLOGY

Transplant Genomics introduced molecular biomarkers TruGraf and TRAC to help clinical teams better understand the risk associated with subclinical rejection. Each of these biomarkers brings an additional piece of the subclinical puzzle to light:

- TruGraf uses gene expression profiling to return a result of TX (immune system quiescence) or non-TX (potential subclinical rejection). The biomarker measures the patient's current immune state and compares it to benchmarks to determine the likelihood that subAR is present.

- TRAC leverages whole genome sequencing to create a donor-derived cell-free DNA quantification. The measurement it returns can help clinical teams understand the likelihood that a graft injury and active rejection is present.

Dr. John Friedewald, MD, FASN, is a Professor of Medicine and Surgery at Northwestern University's Feinberg School of Medicine and a transplant nephrologist who is heavily involved in biomarker technology research. He notes that TruGraf and TRAC help clinical teams identify and respond to acute and subclinical rejection. However, this population only accounts for about 25% of post-transplant patients. The other 75% of patients with stable graft function are still at risk of viruses, which led to continued work on these biomarkers.

HOW GENE EXPRESSION PROFILING AND DD-CFDNA

WORK TOGETHER IN TRAC ID

TRAC ID combines gene expression profiling with dd-cfDNA analysis to enhance post-transplant monitoring by adding a more holistic decision-making ability. A whole genome sequencing process is used that involves:

- **Collecting patient plasma**
- **Isolating nucleic acids within the sample**
- **Optimizing for cfDNA**
- **Performing a library prep sequence and then a whole genome sequence**
- **Isolating human sequences**

This allows teams to understand what portion of cfDNA is donor-derived versus host-derived. In the TRAC assay, a dd-cfDNA of 0.7% or higher indicates a potential for subclinical rejection.

However, the genome sequencing required for the TRAC assay created a robust data byproduct, which Dr. Friedewald said was initially ignored. Isolating human sequences during the whole genome sequencing step left microbial sequences. Dr. Friedewald's team reviewed 2,000 samples from 256 patients to understand whether this metagenomic viral detection could provide value for clinical decision-making in post-transplant care.

VIRUS DETECTION AND ITS ROLE IN GRAFT MANAGEMENT

According to Dr. Friedewald, his team examined 2,000 samples taken over 2 years post-transplant in a clinical trial involving 256 patients. While the trial's original intent was related to the TRAC assay, the data gathered included information about the microbial sequence in the samples. As expected, the team found plenty of common pathogenic viruses in the samples. However, it was looking for something else.

Researchers wanted to find a non-pathogenic virus that was common in most samples. They needed something that didn't cause human disease but would respond to immunosuppression like pathogenic viruses did. They were looking for the metaphorical canary in the coal mine — a virus that could be used as a signal indicator.

Dr. Friedewald points to the torque teno virus (TTV) as that canary. He said that it was present in around 90% of post-transplant patients. In the data Dr. Friedewald's team studied, TTV closely followed the traditional curve of immunotherapy in the 2 years following transplant, indicating that it responded to immunosuppression the same way other viruses did and is, then, an accurate indication of immunosuppression level.

This realization led to the creation of TRAC ID, which adds metagenomic viral detection to these non-invasive biomarker testing options to support a better understanding of patient immunosuppression.

A SPECIFIC TTV USE CASE

To demonstrate the practical application of this new understanding, Dr. Friedewald reviewed TruGraf, TRAC, and TTV data for a patient who also had three biopsies during the 2 years following a transplant. Initially, TRAC scores were high because of post-operative inflammation, but they dropped quickly — as expected. TTV viral load rose, indicating a high amount of immunosuppression — also as expected.

As the patient progressed, the following occurred in the sample data:

- A biopsy was performed around 3 months post-transplant that was normal.
- The patient had instances of BK and CMV infections around the 3-month mark suggesting over-immunosuppression, which led to tapering of immunosuppressive drug doses.
- TTV numbers dropped significantly starting around that time as TRAC and TruGraf results rose — all in response to the reduced immunosuppression.
- At around 15 months, a for-cause biopsy indicated rejection, and TRAC results were 1% dd-cfDNA, also indicating subAR.
- From that time to the 2-year mark, TTV and other data leveled out, and the final biopsy was normal.

Looking only at this data, it's obvious that the patient was initially over-immunosuppressed, leading to infection. The clinical team then likely overreacted, creating under-immunosuppression, which led to the subAR episode confirmed by biopsy. Eventually, the care team figured out the balance, resulting in a healthy graft at the 2-year mark.

However, in presenting this use case, Dr. Friedewald had data about TTV levels and their meaning for immunosuppression that the original team didn't. He points out that if the original team did have that information, they likely could have avoided the rejection by finding the right balance of immunosuppression earlier.

VALIDATION AND CRITICAL PERFORMANCE OF TRAC ID

To understand the clinical validity of cfDNA viral detection, the team looked at existing samples with known viral infections and whether TRAC ID was accurate in identifying them. The team looked at BKV, CMV, EBV, and TTV and found:

Days Prior to the Reported Clinical Infection	Accuracy at Identifying Infection
51-60	40%
41-50	26.6%
31-40	33.3%
21-30	50%
11-20	45%
1-10	91.6%
0	100%

This demonstrates that cfDNA viral detection is fairly precise. Furthermore, it can successfully predict clinical infection almost 2 weeks before traditional methods.

Another analysis examined samples of notable cutoffs associated with BKV, CMV, and TTD. The detection of these viruses via cfDNA was so precise that the analysis picked up positive viral cases before they reached clinically significant thresholds.

Virus	Percent detected	Percent of "clinically significant" cases	Threshhold for clinical significance
BKV	11.5%	8.5%	≥ 1000 copies / mL
CMV	8.3%	4.7%	≥ 1000 IU / mL
TTV	63.7%	26.9%	≤ 104 copies / mL

Dr. Kleiboeker notes that combining metagenomic viral detection and donor-derived cell-free DNA quantification in analyzing plasma from kidney transplants led to high specificity and concordance in detecting viruses. The table below demonstrates that specificity and concordance are well above 90% for all viruses and above 95% for all but JCV.

According to Dr. Kleiboeker, false positive and false negative results in the samples were rare. However, when they did occur, the sample was typically near the lower detection limit for that virus.

Virus	Specificity	Concordance
ADV	98.3%	98.3%
BKV	97.3%	96.1%
CMV	97.6%	97.2%
EBV	99.4%	97.8%
JCV	98.8%	93.9%
TTV	96.6%	98.2%

PERSONALIZED PRECISION MEDICINE: TAILORED POST-TRANSPLANT CARE

Dr. Friedewald provides some hypothetical post-transplant situations to demonstrate how TRAC ID can positively impact care, beginning with a brief look at how TruGraf and TRAC results can guide clinical decisions.

Hypothetical	What it indicates	Recommended action
TruGraf and TRAC are negative	No sign of organ injury or subclinical rejection	Assess immunosuppression status and continue monitoring
TruGraf is positive, and TRAC is negative	No sign of organ injury but the potential for subclinical immune activation	Repeat tests or get a biopsy
TruGraf is negative, and TRAC is positive	Potential subclinical antibody-mediated rejection and organ injury	Repeat tests or get a biopsy
TruGraf and TRAC are positive	A high likelihood of organ rejection	Get a for-cause biopsy

Dr. Friedewald says that around half of all post-transplant patients at any given time will have negative TruGraf and TRAC results. By adding information about the TTV viral load that TRAC ID provides, clinical teams gain access to a more precise decision tree regarding immunosuppression for that population and others.

Hypothetical	What it indicates	Recommended action
dd-cfDNA ≥ 0.7% with high levels of TTV	Potential over-immunosuppression, potential graft injury	Run tests to understand viral loads and conduct a biopsy to rule out allograft injury
dd-cfDNA ≥ 0.7% with high levels of TTV	A potential viral infection, potential graft injury	Run tests to verify infection and consider a biopsy to rule out graft injury
dd-cfDNA ≥ 0.7% with high levels of TTV	Potential under-immunosuppression and alloimmune-mediated graft injury	Consider ruling out graft injury and making changes to immunosuppression accordingly
dd-cfDNA ≤ 0.7% with high levels of TTV	Potential over-immunosuppression	Consider changes to immunosuppression
dd-cfDNA ≤ 0.7% with high levels of TTV	Potential over-immunosuppression	Confirm the pathogen with qPCR and treat it
dd-cfDNA ≤ 0.7% with high levels of TTV	Potential under-immunosuppression	Monitor dd-cfDNA for signs of concerning trends and make changes to immunosuppression accordingly

Dr. Kleiboiker says that TRAC ID can help teams understand the presence of viruses so they can be proactive in managing immunosuppression and graft health.

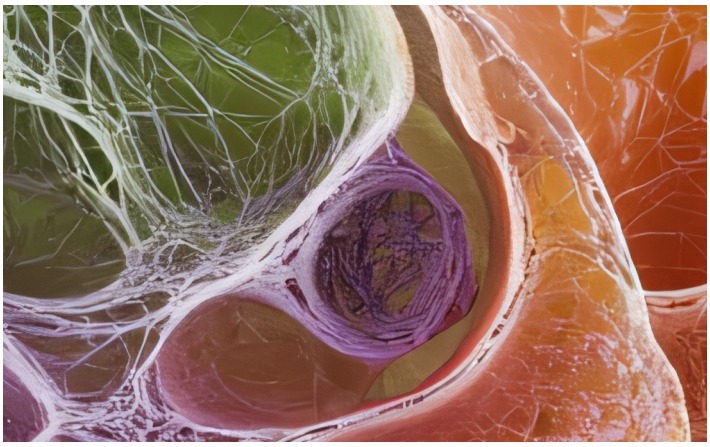
IMPLICATIONS FOR FUTURE TRANSPLANT SERVICES

Precision biomarker integration into post-transplant care offers a number of positivve benefits, including:

- **Cost reductions.**
  TRAC ID and other biomarkers provide non-invasive tools for understanding graft health and immunosuppression, reducing some of the guesswork involved in post-transplant care. When teams can mitigate the trial-and-error approach involved in finding immunosuppression balance for each patient, it may result in substantial cost and time savings.

- **Improved patient outcomes.**
  The ability to identify both subclinical rejection and viral infection earlier and more accurately than with traditional methods allows teams to take a proactive approach to post-transplant care that might reduce episodes of rejection. The insight provided by these biomarkers can also help clinicians educate patients and provide greater peace of mind.

- **Reduced dependency on biopsies.**
  Historically, biopsies have been the golden standard for diagnosing subclinical rejection. However, they are invasive procedures that may cause added stress for patients. Continued work on biomarkers like TRAC ID may help reduce reliance on biopsies.



OPTIMIZING GRAFT SURVIVAL WITH TRAC ID

TRAC ID provides post-transplant clinicians with advanced tools for early detection of clinical rejection and viral infections. When used alongside other tools in post-transplant monitoring, TRAC ID supports a personalized approach to care that can significantly improve positive outcomes.

Ongoing innovation in transplant genomics can build upon these successes to create increasingly accurate testing and better insights for clinical teams. It can also bring these tools into other areas of transplant medicine. Transplant Genomics is committed to such innovation.

Learn more about our work now.